Performance of RTPCR Kits for Reproducible Production of cDNA Libraries

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Introduction

Gene expression studies often rely on quantifying RNA; however, the RNA sample must first be converted into cDNA, a more stable form that is less susceptible to degradation than RNA, through the process of reverse transcription (RT). The enzyme that converts RNA to cDNA, called reverse transcriptase, exists in many different, modified forms.

If the RT process does not result in reproducible cDNA libraries that represent all message species in the RNA extract, the conclusion of gene expression trials may contain errors. Because OSU Forensic studies are researching RNA degradation as a function of storage time for bodily fluid stains, reproducible data is mandatory for accuracy in these experiments.

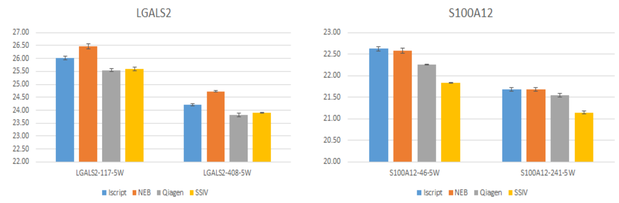
Materials and Methods

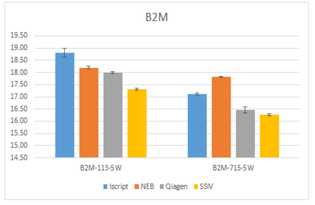
- Complete the RNA extraction from 200ul of a five week old bloodstain using 1000ul of Trizol and 200ul of chloroform.
- Centrifuge, remove clear layer, and filter through the RNA purification column with RNA Prep Buffer and Wash Buffer.
- Each triplicate RNA extract contained 400ng of RNA quantified with fluorescence.
- Several commercially availalble RTPCR kits were evaluated: Qiagen QuantiTech (Q), BioRad iScript (I), Thermo Fischer SuperScript IV (SSIV or S), and New England LunaScript (N).
- In the first RT step converting RNA to cDNA, all RT kits contain random hexamers and oligo dT.
- The abundance of 3 cDNAs present in the cDNA libraries was quantified with qPCR: *LGALS2* (L), *S100A12* (S), and *B2M* (B). These cDNAs represent mRNA transcripts from low to high abundance in the transcriptome. Abundance values are expressed as Ct. (A low Ct represents a higher abundance transcript than one with a high Ct.)

Results

Average of Triplicate Ct Values for Kits

	L117	L408	S46	S241	B113	B715
I	26.03	24.21	22.62	21.68	18.81	17.11
N	26.48	24.73	22.58	21.68	18.19	17.81
Q	25.55	23.82	22.26	21.55	17.99	16.46
S	25.60	23.91	21.84	21.15	17.31	16.27





All kits showed high reproducibility among the triplicates (SD < 0.1 Ct). But overall, SSIV kit gives the highest yield of cDNA, indicated by lower Ct values, and the New England Lunascript kit gives the lowest yield of cDNA, indicated by higher Ct values.

Conclusions

The results suggest that the SSIV RT enzyme has the highest efficiency in producing the most cDNA from an RNA template. Not only does the SSIV kit have the largest yield of cDNA from the RNA template, it also occurs in a short reaction time; a fast enzyme reaction suggests high efficiency in the SSIV modification of RT. On the other hand, this experiment indicates that the least efficient RT modification is the New England Lunascript kit due to its low yield of cDNA.

References

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